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Stavenga, DG; Kinoshita, M; Yang, EC; Arikawa, K

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SHORT COMMUNICATION

D.G. Stavenga · M. Kinoshita · E.-C. Yang
K. Arikawa

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Abstract The regional characteristics of the eyes of butterflies from different families have been surveyed using epi-illumination microscopy, utilizing the eyeshine visible due to the tapetum situated proximally to the rhabdom. All butterflies studied have a high spatial acuity in the frontal region. The facet diameter varies slightly across the eye, and the interommatidial angle and the eye parameter *p* are especially large dorsally. Whereas the ommatidial lattice is generally highly regular, the eyeshine colours distinctly depend on the species. Sometimes the eyeshine is locally uniform, but often it is heterogeneous. It is hypothesized that the regional characteristics as well as the local heterogeneity are adaptations that optimize spectral discrimination.

Introduction

The ommatidia of insect eyes are usually arranged in a strikingly regular lattice, resulting in a crystalline packing of the visual axes of the photoreceptors. As the photoreceptors sample the optical information of the environment, their regular arrangement mediates optimal acuity (French et al. 1977) and thus provides the insect eye with its usually excellent visual capacities.

Gradual changes in the packing density of the ommatidia have created specialized areas with high acuity; e.g. in dragonfly (Horridge 1978), praying mantis (Rossel 1979), butterfly (Land 1989), honey bee drone (Menzel et al. 1991), and male fly *Chrysomia* (van Hateren et al. 1991). In the case of the libellulids and drone bee, the strong eye regionalization is apparent from the different colour of the screening pigments and the large facet size dorsally (Stavenga 1992). Nevertheless, the very regular facet lattice and the uniform colouring seem to suggest that the ommatidial build-up is at least locally identical. Recent research has shown that this concept is inadequate. Although the peripheral photoreceptors of fly eyes (R1–6) are spectrally identical throughout the eye, two types of spectral classes of central photoreceptors (R7/8) exist, that are distributed in a random pattern in the retina (Franceschini et al. 1981; Hardie 1986; Salcedo et al. 1999); the central photoreceptors probably mediate fly colour vision (Fukushi 1989; Troje 1993). A similar random pattern within the spatially crystalline retinal lattice can be recognized in the Japanese papilionid butterfly *Papilio xuthus*, where the spectral characteristics of anatomically identical photoreceptors in adjacent ommatidia can be quite different (Arikawa and Stavenga 1997; Arikawa et al. 1999a, b; Bandai et al. 1992; Kitamoto et al. 1998). Anatomical and molecular biological data demonstrate that the spectral types of photoreceptors in the eye of *Papilio xuthus* co-exist in unique, fixed combinations and that at least three classes of ommatidia can be distinguished. Retinal heterogeneity also has been demonstrated in sphecid wasps (Ribi 1978), moths (Meinecke and Langer 1984) and backswimmers (*Notonecta glauca*) (Schwind et al. 1984), suggesting that heterogeneity is a widespread property of insect eyes.

The design principles underlying the retinal heterogeneity and its consequences for colour vision are presently quite enigmatic. To gain insight into this question we have made a survey of the retinal heterogeneity in butterflies. When suitably illuminated, diurnal butterflies are quite attractive because of the existence of a tapetal reflector basal to each rhabdom (Miller and Bernard 1968). The tapetum is formed by a tracheole folded into a stack of layers, alternately consisting of air and cytoplasm, thus creating an interference reflection filter. This structure mirrors light that has travelled through the rhabdom without being absorbed. The tapetum thus gives the visual pigments in the rhabdom another chance to absorb light. Part of the mirrored light will nevertheless also es-

D.G. Stavenga (✉)
Department of Neurobiophysics, University of Groningen,
The Netherlands
e-mail: stavenga@phys.rug.nl
Tel.: +31-50-3634732, Fax: +31-50-3634740

M. Kinoshita · K. Arikawa
Graduate School of Integrated Science,
Yokohama City University, Japan

E.-C. Yang
Department of Entomology, National Chung Hsing University,
Taiwan

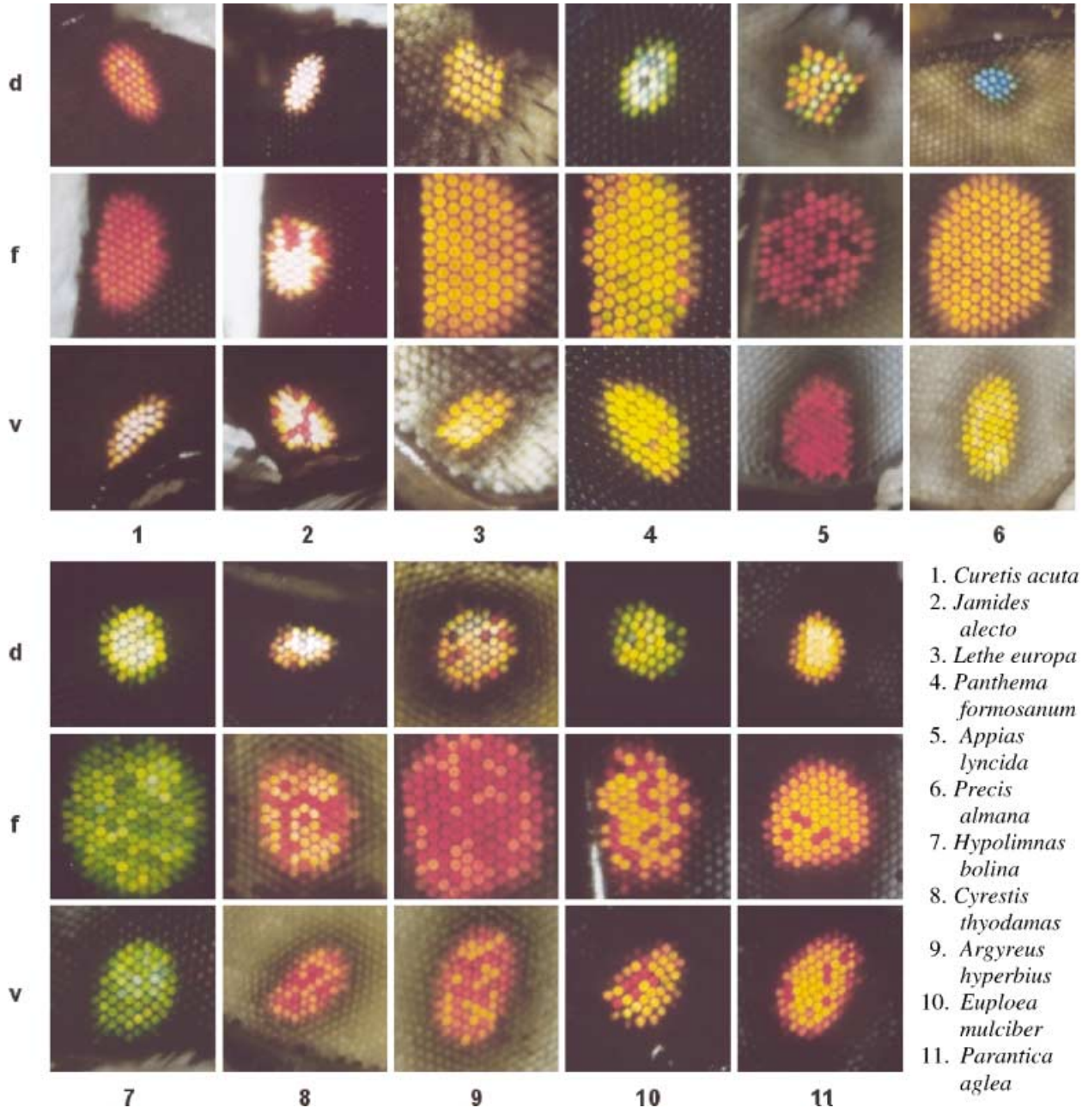

Fig. 1 Photographs of a variety of butterfly species (see also Table 1) in the dorsal (*d*), frontal (*f*) and ventral (*v*) eye regions, respectively. Each figure is 400×400 μm²

cape the second round and leave the eye again. The ommatidial reflectance, called eyeshine, can be inspected by applying epi-illumination (Stavenga 1979). Bernard and Miller (1970), who published a number of photographs of butterfly eyes, showing that the eyeshine can be locally quite heterogeneous, postulated that the tapetal reflector functions to improve colour vision, but progress towards substantiating this claim has not been made since.

Materials and methods

Animals

All investigated butterflies belong to families that have a tapetum proximal to each individual rhabdom. The majority of animals were caught around Taipei, Taiwan; some species were local to Yokohama, Japan. The measurements were performed on live butterflies immobilized by wax and mounted on a platform of a goniometer.

Epi-illumination microscopy

We applied conventional bright-field epi-illumination microscopy using the Olympus metal microscope BX3 M equipped with a 5× Olympus objective, NA 0.10. This numerical aperture is equivalent to an acceptance angle of 5.7°; or, with an interommatidial angle of 2°, eyeshine of roughly 30 ommatidia can be captured. The images of the butterfly eyes were photographed on film (Fig. 1).

Table 1 Colour of eyeshine in butterfly eyes. The *number* preceding some species names corresponds to the number below the photographs in Fig. 1; sex: *m* male, *f* female, – undetermined. The various eye areas investigated are: *frontal* (forward looking direction), *dorsal* and *ventral* (70°±20° upward and downward looking direction, respectively, compared with frontal), and *medial* (area looking in a direction about 30° with respect to frontal). The colours of the eyeshine patterns observed are indicated by capital letters: *B* blue, *G* green, *Y* yellow, *O* orange, *R* red, *P* pale, *K* pink; the lower-case prefixes indicate: *l* light, *d* dark, *v* variable. In each column, the *first mentioned colour* is the dominant one; when the pattern is a mixture of colours, the less dominant colours are also given *in parentheses*

Family, *species*	Sex	Dorsal	Frontal	Ventral	Medial
Lycaenidae					
1. *Curetis acuta*	m	K	vR	vR (K)	vK
2. *Jamides alecto*	f	K (B)	vKO (R)	vO (K, R)	K (R)
Zizeeria karsandra	f	lB (B)	BG (lO, R)	lG (lB, R)	lB (lG, lP)
Megisba malaya	m	lP	lP (lO, R)	lP (lO, lR)	lP (R)
Satyridae					
3. *Lethe europa*	m	vY	O	vO	vO
4. *Panthema formosanum*	–	vG	vO (vG, R)	vYG (R)	vYG (R)
Elymnias hypermnestra	–	vBG	vG (R)	vG (Y)	vG (R)
Ypthima multistriata	–	vBG	vYG	vYG (R)	vYG
Neope muirheadi	m	Y	O	YO (O)	vYG
Pieridae					
5. *Appias lyncida*	m	YG (YR)	vR	R	Y (R)
Appias lyncida	f	vG (O)	R (dR)	vR	R (vG)
Eurema blanda	–	O (dR)	dR (D, R)	R (dR)	R (dR)
Pieris canidia	–	B (G, YG)	O (R, YG)	lR (dR)	O (Y)
Pieris canidia	m			lR (R)	O (R, dR)
Catopsilia crocale	f	R	vR	vR (dR)	vR
Catopsilia pomona	m	vR	R (dR)	vR	vR
Nymphalidae					
6. *Precis almana*	–	B	vO	YG	vO (vG)
7. *Hypolimnas bolina*	m	vYG (BG)	vYG	vYG	vYG
8. *Cyrestis thyodamas*	–	B (O, G)	O (R)	R (O)	R (O)
9. *Argyreus hyperbius*	m	O (R, BG)	R (Y)	R (O)	O (R)
Chitoria chrysolona	m	lY (G)	O	Y (YG)	vYG
Tacoraea selenophora	f	vO	O	vYG	
Lodoga sulpitia	–	vG (BG)	vG (O)	vYG	vG
Kallima inachus	–	vG (YG)	vG (YG)	vBG	vG (Y)
Danaidae					
10. *Euploea mulciber*	–	YG	O (R)	O (R)	vY (YG)
11. *Parantica aglea*	–	Y	O (R)	O (R)	O (R)
Danaus genutica	–	BG (lO, YG)	Y (G)	O (R)	lO (YG)
Idea leuconoe	m	Y	O (R)	O (R)	O (R)
Ideoptis similis	–	Y	O (R)	O (R)	O (R)

Results

We studied the ommatidial reflectance patterns in the eyes of 27 East-Asian (Taiwan and Japan) butterfly species. To make comparison practicable we restricted our inspection to four areas: frontal, corresponding to the forward-looking direction; dorsal and ventral, looking 70±20° upward and downward, respectively, in a plane close to the body symmetry plane; and medial, looking in a direction in the horizontal plane, roughly 30° with respect to the frontal direction. Table 1 summarizes the colours of the eyeshine for a number of selected species as assessed visually, and Fig. 1 presents the appearance in the dorsal, frontal and ventral eye regions, respectively, at the level of the corneal facet lenses.

The facet raster is very regular in most parts of the eyes, except for the occasional lattice error (e.g. Fig. 1, nos 8–11, ventrally). The number of shining facets depends on the eye region. This number is a direct measure of the spatial resolution, because light reflected by the tapetum can be only seen to emerge from ommatidia that have a visual field within the aperture of the objective delivering the epi-illumination. Because the number of shining ommatidia is largest frontally, compared with dorsal and ventral regions (Fig. 1), the interommatidial angle frontally is smallest, or the visual resolution is maximal there. Together with the high resolution, large facet lenses exist frontally, a characteristic correlation shared with most insect eyes. We have so far not detected areas with a specifically high acuity other than frontally in any butterfly.

Table 2 Diameter of facet lens, D, interommatidial angle, $\Delta\phi$, and eye parameter, p

Species	Dorsal			Frontal			Ventral		
	D (μm)	$\Delta\phi$ (°)	p (μm)	D (μm)	$\Delta\phi$ (°)	p (μm)	D (μm)	$\Delta\phi$ (°)	p (μm)
1. *Curetis acuta*	22.3±1.3	1.75±0.13	0.68±0.09	25.2±1.0	1.04±0.05	0.46±0.04	23.8±0.9	2.28±0.46	0.95±0.22
2. *Jamides alecto*	19.5±1.5	2.28±0.46	0.77±0.21	23.3±0.7	1.34±0.08	0.54±0.05	24.9±1.5	1.63±0.23	0.71±0.14
3. *Lethe europa*	29.2±2.2	1.90±0.16	0.97±0.15	33.6±0.8	0.88±0.03	0.51±0.03	29.2±1.1	2.28±0.46	1.06±0.23
4. *Panthema formosanum*	27.3±0.9	1.90±0.16	0.90±0.10	33.7±0.4	0.76±0.05	0.45±0.04	29.7±0.7	1.52±0.10	0.79±0.07
5. *Appias lyncida*	26.8±1.1	1.90±0.16	0.89±0.11	27.9±0.4	0.99±0.04	0.48±0.03	22.7±1.5	1.14±0.11	0.45±0.07
6. *Precis almana*	21.9±1.1	2.28±0.23	0.87±0.13	27.6±0.4	0.81±0.03	0.39±0.02	22.2±3.0	1.27±0.14	0.49±0.12
7. *Hypolimnas olina*	24.0±0.9	1.75±0.13	0.74±0.08	28.5±0.5	0.88±0.03	0.44±0.02	28.6±3.6	1.34±0.08	0.67±0.12
8. *Cyrestis thyodamas*	20.8±1.8	2.07±0.19	0.75±0.13	27.0±0.7	1.20±0.06	0.57±0.05	21.1±2.2	1.52±0.30	0.56±0.17
9. *Argyreus yperbius*	23.8±1.5	1.34±0.08	0.56±0.17	31.9±0.8	0.81±0.03	0.45±0.03	24.9±1.5	1.09±0.05	0.47±0.05
10. *Euploea uliber*	26.0±1.8	1.75±0.13	0.79±0.12	29.2±1.5	0.99±0.09	0.51±0.07	24.7±1.8	1.52±0.10	0.65±0.09
11. *Parantica aglea*	22.1±1.8	1.90±0.16	0.73±0.12	22.7±0.6	1.14±0.06	0.45±0.04	23.2±1.3	1.34±0.08	0.54±0.06

The photographs of Fig. 1 yield the local facet lens diameter, D, and the interommatidial angle, $\Delta\phi$; the latter value follows from the aperture of the microscope objective and the number of shining facets. The values D and $\Delta\phi$ are listed in Table 2, together with the so-called eye parameter, $p=D\Delta\phi$ (Snyder et al. 1977). This is a measure of how close the eye comes to the diffraction limit; its minimal value is 0.25 μm if the light wavelength is 500 nm (for a review, see Land 1989). Comparing the dorsal and ventral eye regions with the frontal area, the lens diameter appears to vary only slightly (less than 25%). The interommatidial angle is invariably smallest in the frontal eye region, yielding the highest acuity there, but it varies strongly over the eye (up to a factor 2.8). The eye parameter is consequently far from constant, especially in the eye periphery (Table 2).

The eyeshine colour varies considerably between species (Fig. 1). The colours can differ to some extent even within one and the same eye, especially the eye periphery compared with the main, central eye regions. Although the large diversity in colours may seem to defy any generality, the dominant colour of the eyeshine is mostly in the long-wavelength range, i.e. yellow and red; with green (e.g. Fig. 1, no 7) in the minority and blue (Fig. 1, no 6 dorsally) being quite rare. A common feature is that the colours of the eyeshine in the dorsal area are of shorter wavelengths than those of the frontal and ventral areas (Table 1).

Discussion

The spatial organization of the eyes of different butterfly species is rather similar. The ommatidial lattice is locally very regular, which is beneficial for optimal spatial acuity (French et al. 1977). Frontally the acuity is invariably highest (Table 2), which correlates with a low eye parameter, predicted for insects active in bright light; the eye parameter values dorsally and ventrally are mostly larger, possibly because of motion blurring (Snyder et al. 1977).

Butterfly eyeshine is light reflected on the tapetum situated proximal to the rhabdom. The observed colour therefore results from the optical system consisting of the spectrally selective reflecting tapetum and the optical-waveguiding rhabdom, which contains the spectrally selective absorbing visual pigments and (possibly) is flanked by screening pigments that act as a spectral filter (Ribi 1979; Stavenga 1979, 1989). A well-studied case is the cabbage butterfly *Pieris rapae*, which has a prominent red eyeshine in the major part of the eye due to short-wavelength absorbing, red-transmitting screening pigment situated adjacent to the rhabdom. Ribi (1979) shows that in the ommatidia of *Pieris rapae* clusters of pigments occur in the photoreceptors near the rhabdom. He treats the pigmentation as being identical in the majority of the ommatidia (except for the dorsal eye part). Recent anatomical and optical studies by X. Qiu, D.G. Stavenga and K. Arikawa (unpublished) show, however, that, similar to *Papilio xuthus*, *Pieris rapae* has three types of ommatidia, and that the different ommatidia are randomly distributed in the retina. The red pigment filter causes a red-shift of the spectral sensitivity of photoreceptors containing green-absorbing visual pigments (Ribi 1979). In *Papilio xuthus* red screening pigment plays a similar role (Arikawa et al. 1999b). We therefore hypothesize that the red reflecting ommatidia of other butterflies have red filtering screening pigments suppressing short-wavelength sensitivity and thus shifting the sensitivity towards the red. The tapetum, which is not present in papilionids (Miller 1979), will further enhance the red sensitivity. The general occurrence of red shining facets then suggests that photoreceptors with sensitivity spectra shifted towards the red occur generally in butterfly eyes (see also Bernard 1979).

The eyeshine colours often vary over the eye (Fig. 1). Retinal regionalization, i.e. a different organization of eye regions, is probably an adaptation for mediating specific visual functions (Stavenga 1992). We therefore presume that the regional spectral differences in butterfly eyeshine reflect adaptations to the spectral characteristics of the light distribution from different directions in the habitat; e.g. the often green or blueish eyeshine dorsally indicates enhanced sensitivity at the shorter wavelengths in the dorsal eye region.

The eyeshine is sometimes locally uniform, but is often heterogeneous. This local heterogeneity is probably not at all detrimentral to vision, as *Papilio xuthus*, which has a prominently heterogeneous eye, possesses excellent spatial and colour vision (Kinoshita et al. 1999). We tentatively conclude that the heterogeneity in the eyes of butterflies and other insects is a widespread adaptation to improve spectral discrimination.

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